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Geographical Variation of Antioxidant Constituent in Garhwal Region of Uttarakhand: Chinese Brake Fern (*Pteris vittata*)

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Abstract

Numerous kind of antioxidants or protecting agent are present in the living body like Glutathione is the master antioxidant produce by the liver and uses free radicals to purify the body. Natural plant consuming antioxidants content is growing the interest for scientific research as well as industrial purposes. Pteridophytes (fern and fern allies) have drawn attention of plants seekers and horticultures since ancient period. Plant of *Pteris vittata* were collected from different geographical region of Uttarakhand Garhwal. The maximum yield of plant extract was found via the ethanol solvent i.e. 5.07-9.67%. DPV extract exhibited a maximum inhibition of 89.32 relatively closed to 91.96% inhibition of Ascorbic acid at the concentration of 0.1 mg/ml by DPPH radical scavenging assay method. The IC_{50} of the DPV extract and Ascorbic acid was found to be 0.543 and 0.495 mg/ml by same. DPV extract showed a maximum inhibition of 72.33 % relatively close to 77.42% inhibition of BHA at the concentration of 0.8 mg/ml by Hydrogen peroxide radical scavenging method. The IC_{50} value of the DPV extract & BHA was found to be 0.279 ± 0.005 mg/ml & 0.257 ± 0.002 mg/ml by same. DPV extract showed a maximum inhibition of 84.32% relatively close to 87.96% inhibition of ascorbic acid at the concentration of 0.8 mg/ml by Nitrogen oxide scavenging method. The IC_{50} value of the DPV extract & BHA was found to be 0.233 ± 0.002 mg/ml to 0.218 ± 0.006 mg/ml by same.

Keywords: Chinese fern; Antioxidant; Scavenging; Phenolic compound; Inhibition

Introduction

Natural antioxidants are required either prevent or cure the disorders caused by free radicals. As folk medicine, the Pteridophytes which constitute fern and ferns allies, have been known to man for more than 2000 years, and also been mentioned in ancient literature [1]. Pteridophytes are original vascular cryptogams, which flourish well in worldly environment. In the world flora of pteridophytes 12,000 species has been identified among which 1,000 species into 70 families and 191 genera are occur in India. *Pteris vittata* was also entitled as the Chinese brake, Chinese ladder brake, or simply ladder brake [2,3].

Pteridophytes is predictable for the rich diversity of valuable antioxidants. Various works was accomplished towards the medicinal importance of pteridophytes by the researchers. Antioxidants are the chemical which are derived from the plant sources, generally affect health, but are not yet established nutrients. Phenolic rich content plant material simultaneously increasing the interest of market as antioxidant used in the food industry. These natural antioxidants improve the quality and nutritional value of food. Flavonoid related derivatives have remarkable antibacterial, antiviral, anti-inflammatory, anticancer and anti-allergic activities due to its scavenging activity [4].

The investigation was designed to explore the variation of the antioxidant content of whole plant of Chinese Brake, in the different region of Uttarakhand.

Material and Methods

Plant material

The investigation was conducted out in the month of August-September, 2018 at Uttaranchal Institute of Pharmaceutical Sciences, Premnagar, Dehradun, Uttarakhand. The fresh whole plant was collected through habitat of Uttarakhand i.e. Chamoli, Dehradun, Pauri, Tehri and Uttarkashi. The herbariums of plant were submitted to the Forest Research Institute, Dehradun, Uttarakhand. The plant was sundried for 15-20 days in the laboratory and transformed into powder.

Preparation of plant extract

Air dried plant powder was softened in petroleum ether & stored in tightly closed container. It was rotated on a rotary shaker at 190-220 rpm for one day & the clear filtrate was discarded. Petroleum ether was fully evaporated & collects as powder. Further the powder was extracted out by the different solvent acetone, benzene, ethanol, ethyl acetate and water respectively. By the centrifugation techniques, the resulting extract was obtained as dry residues [5]. All chemical & solvent used during the experiments were analytical grade. The plant extract were entitled consequently as their localities i.e Chamoli *P. vittata* (CPV), Dehradun *P. vittata* (DPV), Pauri *P.* vittata (PPV), Tehri *P. vittata* (TPV) and Uttarkashi *P. vittata* (UPV).

Qualitative phytochemical analysis

Highest yield of extract was extracted in the ethanol solvent relatively to others. Therefore ethanol extract was subjected to preliminary qualitative phytochemical investigation for alkaloids, proteins, carbohydrates, flavonoids, cardiac glycosides, saponins, catechins, sugars steroids and triterpenoids, tannins and phenols, etc., [6].

Quantitative phytochemical analysis

The phytochemicals constituent was enumerated by the standard procedures.

Determination of total alkaloids (TAS)

1 gm of extract was liquefied in 40 ml of 10% acetic acid in ethanol

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& strained. It was concentrated & add conc. ammonium hydroxide drop wise for the precipitation. The precipitated material was further liquefied with dilute ammonium hydroxide & again concentrated. The resulting residue was alkaloid [5].

Determination of total saponin content (TSC)

2 g of extract liquefied in 20% of aqueous ethanol. The samples was heated for 90 minute & strained out. This procedure revised atleast three times. The resulting solution dissolved with ether, shaken vigorously. The saponin content was recovered by aqueous layer carefully through the mixture of n-butanol & 5% sodium chloride solution. The saponin content was weighed [7].

Estimation of total phenolic content (TPC)

The total phenolic content was estimated by standard procedures with slight modification [8]. 2.5 mg of plant sample was dissolved in 10 ml of ethanol & sonicated. There was incubated the solution of plant sample, methanol, and distilled water & Folin-Ciocalteu reagent for 5 minute. Add the 10% sodium carbonate solution & covered through aluminum foil. It was again incubated for 20 min. The absorbance of the sample was determined using a UV visible spectrophotometer at 765 nm. Gallic acid is used as standard phenolic compound. 100 µg/mL solution of gallic acid was prepared by dissolving 10 mg of Gallic acid monohydrate in 100 mL of ethanol. By diluting the stock solution, obtained different concentration ranging from 10-80 µg/ml. Total phenolic content was stated as µg of gallic acid equivalents (GAE) per ml. A standard calibration curve was drawn by plotting absorbance against concentration. It was found to be linear over this concentration range.

Estimation of total flavonoid content (TFC)

Aluminium chloride technique was slightly modified for the flavonoid estimation [9,10]. Prepared the mixture of sample solution, methanol, 10% aluminium chloride, 1 M potassium acetate solution and distilled water in test tube & incubated for 30 minutes. The reaction mixture was treated with 1M sodium hydroxide solution. Rutin is used as standard flavonoid compound. 10 mg of rutin dissolved in 10 mL of ethanol to get 100 µg/mL. By diluting the stock solution, obtained different concentration ranging from 10-80 µg/ml. The total flavonoid content was expressed in microgram of rutin equivalents (RE) per gram of samples. The absorbance was measured at 415 nm against reagent blank. The calibration curve was drawn by plotting absorbance against concentration. It was found to be linear over this concentration range.

DPPH radical scavenging activity

The DPPH radical scavenging activity was performed by standard procedures with slight modifications [11,12]. DPPH (1, 1-Diphenyl –2-picrylhydrazyl) is a kind of stable free radical. Stock solution was prepared the concentration of 0.43 mg/ml. Ascorbic acid was used as standard. Prepared the solution of DPPH with sample & control separately. The control contained 0.1 ml ethanol in place of the plant sample. The absorbance was measured at 517 nm by UV spectrophotometer [8].

Antioxidant activity was expressed as percentage

% Antioxidant activity = [(Ac – As) /Ac] × 100

Ac and As are the absorbance of control and sample, respectively. The IC_{50} was calculated for sample & control by Figure 1.

Hydrogen peroxide scavenging activity

The hydrogen peroxide scavenging assay was carried out by standard procedure with minor modification [13]. Hydrogen peroxide is an example of oxidizing agent. H_2O_2 (6%v/v) was prepared in phosphate buffer (0.1M, pH 7.4). Butylated hydroxyl anisole (BHA) was used as standard. The different concentration was prepared in the phosphate buffer & hydrogen peroxide solution. The absorbance was recorded at 230 nm by UV spectrophotometer. Scavenging activity was expressed as percentage inhibition

H_2O_2 scavenging activity (%) = (A0 – A1) /A0 ×100

A0 is the absorbance of the control, and A1 is the absorbance of the sample. The IC_{50} was calculated for sample & control by Figure 2.

Nitric oxide scavenging activity

The NO scavenging assay was carried out by the use of Griess Illovay reaction [14]. Griess reagent was mixture of 0.1% sulfanilamide, 0.2% phosphoric acid & 0.01% N-(1-naphthyl) ethylene diamine dihydrochloride). Aqueous solution of sodium nitroprusside generates nitrogen oxide (NO) at pH=7 & interact with oxygen molecule to produce stable products (nitrates & nitrite). Sodium nitroprusside in phosphate buffered saline was mixed with different concentration of sample, then dissolved in methanol & incubated at 40°C for 90 minutes. Control was prepared without sample. After the incubation period, add 0.1 ml of griess reagent. Ascorbic acid was used as standard. The absorbance was calculated at 540 nm.

Scavenging activity was expressed as percentage inhibition

NO scavenging activity (%) = (A0 – A1) /A0 ×100

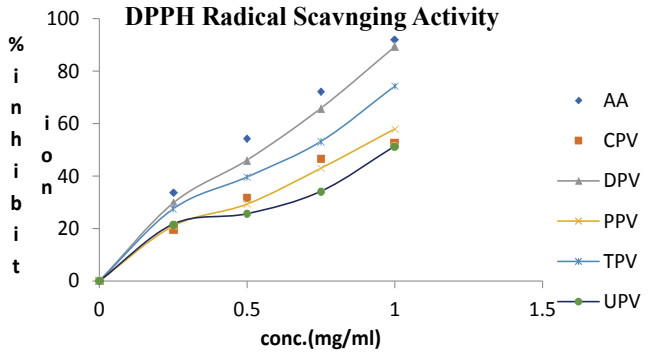

Figure 1: DPPH radical scavenging (%) inhibition versus concentration (mg/mL) of standard and extracts.

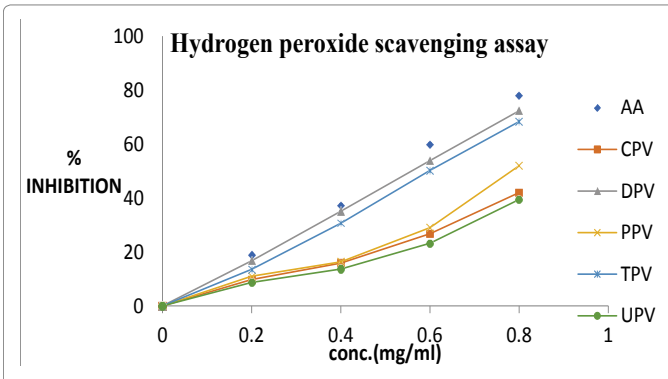

Figure 2: Hydrogen peroxide scavenging (%) inhibition versus concentration (mg/mL).

A0 is the absorbance of the control, and A1 is the absorbance of the sample.

Results

Yield and phytochemical estimation of crude extracts

Ethanol extracts of *P. vittata* was appeared as green to dark green. Phytoconstituent was found to be in range of 7.37-.9.67%. Plant extract was exposed the different qualitative test & revealed positive for certain phytochemical tests. Alkaloids, phenolic compounds, flavonoids, saponins and tannins were the secondary metabolites, present in the extract. These compounds play significant role of antioxidant activity of natural resources. Variation in the amount of these constituents directly influences the antioxidant activity of compound [15].

Total alkaloid content and total saponin content

Alkaloid content of CPV, DPV, PPV, TPV & UPV was found to be in the range of 0.819 ± 0.053, 0.152 ± 0.40, 0.919 ± 0.906, 0.115 ± 0.3 & 0.831 ± 0.628 µg /g respectively. Saponins content of CPV, DPV, PPV, TPV & UPV was found to be in the range of 0.595 ± 0.71, 0.119 ± 0.185, 0.737 ± 0.503, 0.817 ± 0.125 & 0.541 ± 0.528 µg/g respectively. Among all of extract, DPV and TPV revealed the maximum content of alkaloid and saponins. Alkaloids are the widely available phytochemicals responsible for the anticancer and antimicrobial activities. Saponins are applicable as a suitable option for phytochemicals to defend plant against several pathogens [16].

Total phenol content (TPC)

TPC was calculated from the calibration graph of gallic acid verses sample. Multiple reading was recorded for each sample. The TPC was revealed to be in the huge variation range of 0.411 ± 0.327 to 1.653 ± 0.479 mg GAE/g (Figure 3). Linear regression analysis (y=0.0163x+ 0.0083, R^2=0.0997) was applied to calculate TPC in which y is absorbance at 765 nm and x is the amount of gallic acid equivalent (g/ml) per 20 gm extract.

DPV extract was found the highest amount of TPC i.e. 1.605317 ± 0.4794 mg GAE/g. Hydroxyl group of phenol play important role in the scavenging activity of plant [17,18].

Total flavonoids content (TFC)

TFC was calculated from the calibration graph of rutin verses sample. Multiple reading was recorded for each sample. The TFC was revealed to be in the huge variation range of 0.346 ± 0.89 to 0.714 ± 0.006 µg RE/g (Figure 4). Linear regression analysis equation (y=0.015x+0.026, R^2=0.9994), was applied to calculate TFC in which y is absorbance at 415 nm and x is the amount of rutin equivalent (RE) per 20 g extract.

DPV extract was found the highest amount of TFC i.e. 0.7143 ± 0.0064 µg RE/ g. Like phenolic, flavonoids have positive effect on human health [19].

DPPH radical scavenging activity

Ascorbic acid & antioxidant content present in the extract directly react with DPPH radical & produced a yellow diphenyl- β-picryl hydrazine complex. Deviation of discoloration directly proportional to phenolic & flavonoid content (Figure 1). DPV extract was showed a maximum inhibition of 89.32% relatively close to of 91.96% inhibition of ascorbic acid at the concentration of 0.1 mg/ml. IC_{50} of the DPV extract & ascorbic acid was found to be 0.543 ± 0.002 mg/ml & 0.495 ± 0.0005 mg/ml. Percentage inhibition of other was found to be in range

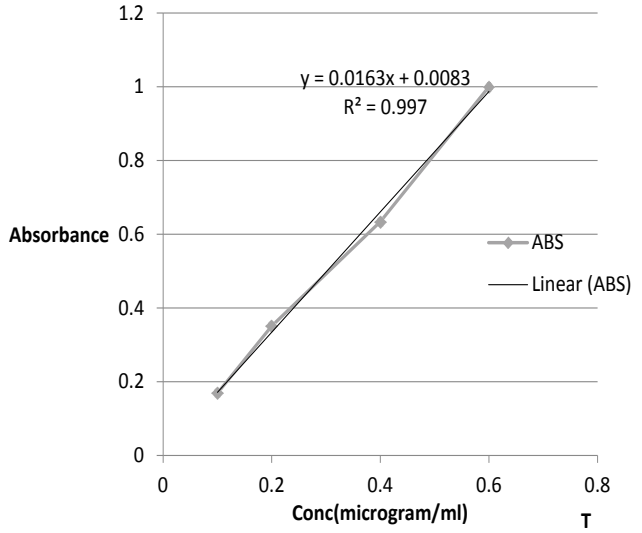

Figure 3: Standard curve between concentration of Gallic acid (µg/ml) and absorbance.

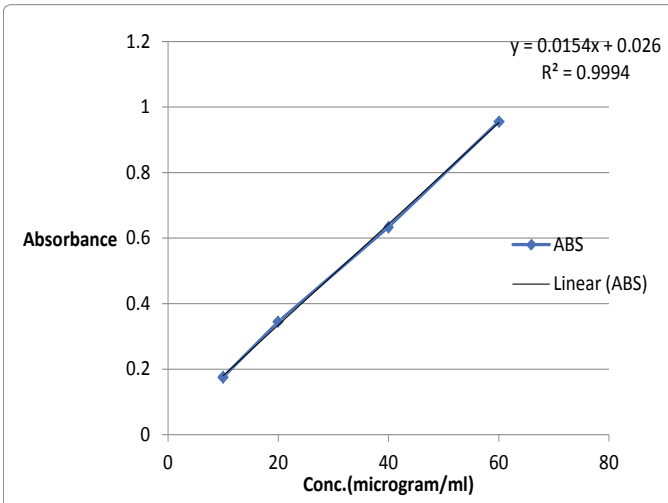

Figure 4: Standard curve between concentration of Rutin (µg/ml) and absorbance.

of 51.44 to 74.33%. IC_{50} of other was found to be in the range of 0.871 ± 0.001 to 1.006 ± 0.007 mg/ml [20,21].

Hydrogen peroxide scavenging activity

Hydrogen peroxide is oxygen containing species involved in the certain cellular activities like phagocytosis, cell growth & synthesis of biological compound. Hydrogen peroxide can cross cell membranes rapidly [22]. Hydrogen peroxide scavenging activity of the extract are present in Figure 2. DPV extract showed a maximum inhibition of 72.33% relatively close to 77.42% inhibition of BHA at the concentration of 0.8 mg/ml. The IC_{50} value of the DPV extract & BHA was found to be 0.279 ± 0.005 mg/ml & 0.257 ± 0.002 mg/ml. Percentage inhibitions of others were found to be in the range of 39.44 to 68.33%. IC_{50} of others were found to be in range of 0.301 ± 0.001-0.553 ± 0.001 mg/ml.

Nitric oxide scavenging activity

Nitric oxide is potent chemical mediators which regulate the certain

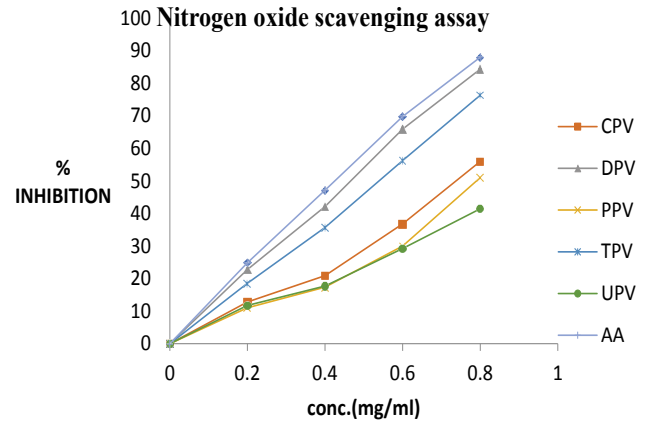

Figure 5: Nitric oxide scavenging (%) inhibition versus concentration (mg/ml).

activities like smooth muscle relaxation, neuronal signaling, inhibition of platelet aggregation and regulation of cell mediated toxicity etc. It is a diffusible free radical [22]. The scavenging of nitrogen oxide is present in Figure 5.

DPV extract showed a maximum inhibition of 84.32% relatively close to 87.96% inhibition of ascorbic acid at the concentration of 0.8 mg/ml. The IC_{50} value of the DPV extract & BHA was found to be in the range of 0.233 ± 0.002 mg/ml to 0.218 ± 0.006 mg/ml. Percentage inhibitions of others were found to be in the range of 41.44%-76.33%. IC_{50} of others were found to be in the range of 0.259 ± 0.0052 to 0.499 ± 0.001 mg/ml.

Conclusion

Uttarakhand is rich source of natural sources. Alkaloid, saponins, phenolic and flavonoid constituent are possessing efficient antioxidant activity in natural resources. It is unfortunate that these forms were discounted group of plants in biodiversity, in spite of their economic value is familiar in worldwide. We summarized the outcomes that antioxidant activity of chinese brake fern was strongly revealed by ethanol extract of Dehradun & Tehri district. It is very much supportive in the era of new drug investigation for the cancer & tumors disease. Therefore cultivation should be supported as a medicinal plant for the development of health care products for aging and chronic disease. The plant extract was obtained from different graphical region showed the strong presence of natural antioxidant. It is concluded that Dehradun and Tehri District possessing more favorable condition for the cultivation of Chinese bracken fern. This work will provide a foundation for further isolation of natural antioxidant constituent especially Dehradun and Tehri district of Uttarakhand.

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Conflicts of Interest

The authors declare there is no conflict of interest.

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